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PHYSIOLOGICAL BEHAVIOR OF ENZYMES AND CARBOHYDRATE TRANSFORMATIONS IN AFTER-RIPENING OF THE POTATO TUBER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 148

CHARLES O. APPLEMAN

Introduction

An active growing period, followed by a period of rest, is a very general phenomenon among plants. Thus most seeds, tubers, and bulbs are structures whose growth processes have been arrested. Germination is simply a continuation of growth after the awakening from the rest period. The buds of the potato tuber, for example, will not grow, under the most favorable conditions, for several weeks after maturity; but after the awakening from this dormant condition they will grow under much less favorable conditions in a cold cellar.

Certain changes occur during the apparent dormancy, which are antecedent to the release of the growth processes, and these changes are known as "after-ripening." In many seeds the rest period is due to coat characters, which exclude or limit the supply of water or oxygen. After-ripening in such cases are processes which render the coats permeable to these substances. Often the processes of after-ripening are metabolic in character; this is especially true of tubers.

Certain external agencies artificially applied have been found to accelerate the processes of after-ripening, and thereby greatly shorten the natural rest period. MÜLLER-THURGAU (1) has shown that exposure to 0° C. for one month has this effect upon the resting potato tuber. Changes are thus brought about which break the rest period and allow growth to proceed several weeks earlier than under ordinary circumstances. He also found a great reduction of respiration, accompanied by an accumulation of sugars during exposure at low temperature.

The following work is a further study of the metabolic changes occurring in the potato tuber as after-ripening proceeds during storage at 0° C. It is intended to throw some light upon the nature of the limiting factor or factors of growth during the rest period of this particular organ. Thus far a quantitative study has been made of digestive and respiratory enzyme changes and the carbohydrate food transformations.

Material

The material used for this investigation was the red land potatoes grown in Texas and procured only a few days after being harvested. This variety has a very short rest period. Two crops a year are grown in this climate, and samples from both crops were used in this work. The tubers were wrapped with paraffin paper and one-half stored at 0° C. and the remainder stored in a dark basement room at 20–25° C.

Methods

GLUCOSE, SUCROSE, AND STARCH.—The methods employed for the determinations of glucose, sucrose, and starch were virtually those of the association of official agricultural chemists (**7**). The glucose was determined according to the method of MUNSON and WALKER.

DIASTASE.—A modification of the starch iodine method (**8, 9**) was used in making the diastase determinations. One cc. of a 1 per cent soluble starch solution was placed in each of 10 test tubes surrounded with ice. Ascending amounts of the potato extract, prepared by grinding with quartz sand and filtering, were added to the tubes, beginning with 1 cc. and increasing the amount 0.1 cc. for each succeeding tube. The tubes were then incubated at 40° C. for 48 hours, placed again in ice, filled nearly full with water, and 3 drops of iodine solution added to each tube. The first tube in the descending series which showed a blue or violet color was considered the index for comparative diastatic activity. The method is impractical with larger amounts of extract on account of dark oxidation products and the precipitate which falls on addition of the iodine. If small amounts of both starch solution and extract are used, and the incubation continued for

48 hours, satisfactory duplicates may be obtained, so the method was considered sufficiently reliable for comparative purposes. A few drops of toluol were added to each tube during incubation, to insure against activity of bacteria.

CATALASE.—A detailed description of the method for the catalase determinations will be found in a recent paper by the author published in this journal (**4**). In all cases the water bath temperature was 25° C. during the experiment.

PEROXIDASE.—In order to compare the rate of peroxidase activity in the material under investigation, various methods were tried and variously modified. The methods differed mainly in the preparation and treatment of the extract, for in all cases guaiaconic acid was used as the oxidizable substance (**10, 11**). A definite quantity of extract was allowed to act upon a definite quantity of guaiaconic acid in the presence of hydrogen peroxide. The time required for the oxidation of the guaiaconic acid to reach a depth of blue equal to that of a standard tube, containing a solution of indigo carmine, was considered the index of the peroxidase activity. The standard tube was placed against a white background and screens arranged to eliminate shadows and unequal lighting. If the test tube is held against the standard tube, and the eye fixed upon the line separating the tubes, and the time noted when the color is the same on both sides of the line or when the line seems to disappear, quite accurate readings may be obtained with a little practice. The error of reading is greatly reduced if the test solution is of such dilution or the standard tube of such depth of blue that the time of reaction is not less than ten seconds nor greater than one minute. If the solution of indigo carmine is made up with pure distilled water, the fading of color at the end of several weeks is so slight that it falls well within the experimental error involved in comparing the shades of blue.

The present methods for peroxidase determinations are unsatisfactory, but under careful manipulation the above method will give results of value for determining comparative activity of the guaiaconic acid oxidizing peroxidase in potato tubers.

The method described by GRÜSS (**2**) for obtaining a peroxidase solution from the potato tuber was first tried. According to this method, morphologically similar parts of the tuber were sliced directly into absolute alcohol and heated to 70° C. for 10 minutes in order to destroy the oxidases. The slices were allowed to remain in alcohol 24 hours. The alcohol was changed three times. They were then dried between filter paper, covered with ether for a few minutes, replaced between filter paper, quickly dried in a vacuum desiccator, and thoroughly pulverized in a mortar. One gram of this powder was ground one minute with quartz sand and 25 cc. water and filtered. For the tests 5 cc. of the filtrate were used and 0.5 cc. of guaiaconic acid solution and 0.1 cc. of one-half per cent hydrogen peroxide. The guaiaconic acid solution was prepared by dissolving 0.5 gram of guaiaconic acid in 50 cc. alcohol.

Three sources of error were soon discovered in this method of extract preparation, which render it unreliable for comparative purposes. Filtering, which is necessary in order to obtain a sufficiently clear solution for colorimetric work, removes a large percentage of the peroxidase. This is probably due to inclusion of the peroxidase in the clot of coagulable proteins, and not to an insoluble form of the enzyme. Filtering has no effect on the peroxidase activity of fresh unheated extract.

TABLE I

EFFECT OF FILTERING ON PEROXIDASE ACTIVITY OF HEATED EXTRACT

HEATED TO 70° C. for 10 MIN.	SECONDS REQUIRED FOR REACTION TO REACH STANDARD TUBE		
	Unfiltered	Filtered through cotton	Filtered through paper
Sample 1	..	28	56
Sample 2	..	12	25
Sample 3	15	..	45
Sample 4	15	..	35

Another source of error probably arises also from the presence of coagulable proteins. The peroxidase leaches out of the coagulum and thereby renders the peroxidase activity of the solution very unstable, as shown in table II. HASSELBRING and ALSBERG (5) found a similar condition in studies upon oxidases, and first concluded that it must be due to coagulable proteins.

It is not due to activation of zymogen, as the phenomenon does not occur in the fresh unheated extract method described later.

TABLE II

Shows increase in peroxidase activity of solution on standing

	Seconds required for reaction to reach standard tube		
		After 2 hours	After 24 hours
Slices of potato dehydrated with alcohol and ether; heated to 70° C. for 10 min.	51	34	30

A third source of error, which would have to be taken into account, is the degree and time of drying. If the slices are dried in a desiccator for several hours, the peroxidase activity is greatly impaired. After a few days the peroxidase is practically destroyed in the powder produced by grinding the dried slices (table III). Gortner (6) has recently described a tyrosinase, which loses its vitality on drying.

TABLE III

Shows loss of peroxidase in potato with drying

Potato tuber	Time required for reaction to reach blue of standard tube
Slices dried in desiccator 30 min.	24 seconds
Slices dried in desiccator 22 hrs.	120 seconds
Powdered slices standing 4 days exposed to laboratory air	10 minutes

The above facts alone are sufficient to render the method described by Grüss of little value for determining peroxidase activity in conditions approaching those of the living tuber. By grinding the potato tuber with $CaCO_3$, thus neutralizing the acids, the peroxidase activity was found to be quite stable for some time in the resulting extract (table IV).

Based upon this fact the following procedure was employed for the final comparative determinations of the peroxidase in the potato tubers under investigation. The tubers were grated with frequent dipping of the surface in $CaCO_3$. The pulp was then ground in a mortar with quartz sand for two minutes, and the extract pressed through cotton and cheesecloth; 1 cc. of the extract was at once added to 300 cc. of water. This dilution gave a solution sufficiently clear for the test and a speed of reaction which reached the standard color in less than a minute. It was unnecessary to destroy the oxidase by heating. In this dilute solution the time required for the oxidase to produce a visible blue was longer than that required for the peroxidase to produce the blue of the standard color.

TABLE IV

EFFECT ON PEROXIDASE ACTIVITY OF EXTRACT WHEN THE POTATO TUBER IS GRATED WITH $CaCO_3$

POTATO TUBER	SECONDS REQUIRED FOR REACTION TO REACH BLUE OF STANDARD TUBE		
		After 6 hours	After 24 hours
Ground with $CaCO_3$	21	21	30
Ground without $CaCO_3$.....	45	..	90

OXIDASE.—Colorimetric methods, as well as the direct measurement of the oxygen consumed by the oxidation of hydrochinon in the manner recommended by GRÜSS (2), were used in an effort to ascertain the comparative rate of oxidase activity. So many sources of error were discovered in applying these methods to the material under investigation, that the results were considered too unreliable for publication. Better methods than those in present use are much needed.

Experiments

In comparing results obtained by the two methods for peroxidase determinations previously described, it was found that the alcohol and ether method always gave a much greater peroxidase activity in the cold storage potatoes than in those stored at room temperature, while the fresh extract method gave practically the same in both cases during the same period of storage. According to the following table this would seem to be due to an alteration at low temperature of coagulate protein, which modifies the amount of peroxidase occluded by clot, for after 24 hours of leaching the activity is just as great in the room-stored potatoes.

TABLE V

SHOWS INCREASE IN PEROXIDASE ACTIVITY IN COLD STORAGE POTATOES BY THE ALCOHOL AND ETHER METHOD, BUT NO INCREASE WHEN THE TESTS ARE MADE WITH FRESH UNHEATED EXTRACT

POTATO TUBER	SAMPLE	SECONDS REQUIRED FOR REACTION TO REACH STANDARD COLOR			
		Storage at 20°–25° C.		Storage at 0° C.	
			After 24 hours		After 24 hours
Slices treated with alcohol and ether; heated to 70° C. in alcohol for 10 minutes	1	60	20	25	20
	2	56		21	
	3	51		32	
Ground with $CaCO_2$ and determinations made with fresh unheated extract	1	35	42	35	40
	2	31		30	
	3	32		25	

The following table is a summary of the metabolic changes thus far studied occurring in a typical lot of young potatoes stored at 0° C. and 20–25° C. for a period of 6 weeks, tests being made every 2 weeks. Several lots were run, but as they all showed practically the same changes, only a typical table is given.

TABLE VI

SHOWS METABOLIC CHANGES OCCURRING IN POTATO TUBERS DURING AFTER-RIPENING AT LOW TEMPERATURE

EXPOSURE	TEMPERATURE	GLUCOSE	SUCROSE	STARCH	DIASTASE	CATALASE	PEROXIDASE
Days	° C.	Per cent	Per cent	Per cent	cc. potato extract to digest 100 cc. starch sol. in 24 hrs. at 48° C.	cc. of O_2 evolved in 3 min.	Seconds required to reach standard color
14	20–25	0.37	0.55	14.4	30	40.6	35
	0–+1	0.52	1.3	13.2	25	37.6	35
28	20–25	0.32	0.54	14.	30	41.2	33
	0–+1	1.8	1.65	11.	20	28.6	32
42	20–25	0.3	0.84	13.6	25	44.	32
	0–+1	3.5	1.8	9.6	20	26.4	25

Potatoes stored at 0° C. or below exude an acid fluid. This fact suggested the possibility that the increased diastatic activity after cold storage may be due to free acids. The decreased catalase activity might also be thus accounted for. The following single experiment is evidence in favor of this view.

Two potatoes of nearly equal weight were grated and thoroughly ground, one with $CaCO_3$, and the other without. Both were stored at 0° C. for 34 days, and analysis made according to table VII.

TABLE VII

EFFECT OF STORAGE AT 0° C. FOR 34 DAYS WHEN POTATO IS PREVIOUSLY GROUND WITH AND WITHOUT $CaCO_3$

POTATO GROUND	GLUCOSE	DIASTASE	PEROXIDASE	CATALASE
	Per cent	Units of extract to digest a unit of starch	Seconds required to reach standard color	cc. of O_2 evolved in 3 min.
Without $CaCO_3$	0.18	100	30	0.1
With $CaCO_3$	trace	160	40	6.7

The expressed potato sap is strongly acid. After repeated titrations, I found that the sap from cold storage potatoes and that from room-stored ones contained practically the same amount of acid. Low temperature, therefore, does not change the total acidity, but probably affects the permeability of protoplasmic membranes, and thereby allows the acids to reach zymogens more rapidly than under normal conditions.

Conclusion and summary

The method recommended by GRÜSS for determining the rate of peroxidase activity in the potato tuber gives no indication of the rate of activity in conditions approaching those of the living tuber. This is due to errors introduced by inclusion of the peroxidase in the clot of coagulable proteins, and to the loss of peroxidase during the process of drying the slices. After a few days the peroxidase is practically destroyed in the dry powdered potato. This method always showed an apparent increase in peroxidase activity in cold storage potatoes. The evidence at hand seems to indicate that this is due to an alteration of coagulable proteins by low temperature, thus modifying the amount of peroxidase occluded by the clot.

Certain internal changes are accelerated by 0° C., which shorten the rest period of potato tubers.

Both glucose and sucrose accumulate. The increase in sucrose is more rapid at first than glucose, but by the end of 6 weeks of storage at low temperature the percentage of glucose is about twice that of sucrose.

Diastase activity was greater in the cold storage tubers than in those stored at room temperature at the end of 2 and 4 weeks; but at the end of 6 weeks there was no appreciable difference, as the variety used for this work was near the end of the rest period. A few had already germinated. The increased diastase activity is probably due to greater activation of zymogen by free acids, which are liberated by the greater permeability of protoplasmic membranes at low temperatures.

Catalase is very abundant in potato tubers stored either at 0° C. or at room temperature, but suffers a gradual reduction as storage at 0° C. continues. The presence of free acids would cause this reduction, as catalase is rapidly destroyed by the free acids in ground potato pulp. This behavior of catalase corresponds with that of respiration under similar conditions, a significant fact in the light of a recent claim (**3**) that catalase is the primary factor in alcoholic fermentation, and therefore probably in respiration.

A guaiaconic acid peroxidase is very active in potato tubers at the beginning of the rest period and increases slowly as the end of the rest period approaches. Low temperature had no appreciable effect in hastening this increase in the material used for this work, according to the method employed for its determination.

The changes peculiar to after-ripening may be in the buds, and the metabolism of the tuber as a whole may bear little or no causal relation to these processes.

The writer wishes to acknowledge his indebtedness to Dr. WILLIAM CROCKER for suggesting the problem and for his untiring interest and assistance during the progress of the work.

AGRICULTURAL EXPERIMENT STATION
COLLEGE PARK, MARYLAND

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